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C Wassmer 9/9/97

PI - Signature Date

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## Introduction

The following is a progress report for Grant No. DAMD17-94-J-4176 entitled, "Radioimmunotherapy of Metastatic Breast Cancer Using Radiolabeled Tumor Specific Antibodies" for the period of September 1, 1996 to August 31, 1997.

The overall goal of the project is to develop effective therapy for breast cancer using radioimmunoconjugates. As noted previously, the humanized version of BrE-3 (huBrE-3) designed at the Cancer Research Fund of Contra Costa by Drs. Joseph Couto and Roberto Ceriani in collaboration with E. Padlan (NIH) has become available to us. The purpose of the design was to diminish the immunogenicity of the frameworks while strictly maintaining the antigen binding affinity.

A strategy aimed at increasing the potential efficacy of radioimmunotherapy is by combining this with other agents such as chemotherapy. As noted in my previous progress report, at NYU, a model drug that we have helped develop is the topoisomerase-1 inhibitor topotecan. Topoisomerase-1 is a nuclear enzyme involved in unwinding of supercoiled DNA and is integrally involved in a host of cell functions including replication and transcription. In the presence of topotecan, there is a stabilization of the complex formed by topoisomerase I and DNA, preventing the religation of the DNA strand. Interaction between the stabilized terniary complex and the replication fork is thought to convert single strand breaks into double strand breaks and cause cell death. Topotecan interaction with this enzyme converts topoisomerase-1 into a "cellular poison" and results in progressive cell death.

We have conducted a Phase I study utilizing a novel schedule for administration of topotecan, under sponsorship of CTEP. In this study topotecan was given as an ambulatory infusion in low doses, continuously for up to a 21-day duration. We have determined the MTD for heavily pretreated patients to be 0.53 mg/m2/day for 21 days, increasing dose intensity by >50% compared to the conventional (daily x 5) administration schedule. We have also observed unprecedented activity in a phase I study, including partial remissions in patients with ovarian and breast cancer (previously received 4-5 regimens) and renal cancer (1). We are currently completing a phase I study evaluating 3 hour paclitaxel and 14 day continuous-infusion topotecan in patients with advanced cancer.

While studies of topo-1 inhibitors in combination with radioimmunotherapy have not yet been reported, experimental models with external beam radiation therapy show that the combination of these two modalities enhance cell kill in cell culture and *in vivo* (2-6). It has been postulated that the synergism between the topo-1 inhibitors and ionizing radiation is due to the ability of topo-1 inhibitors to interfere with repair of radiation-induced DNA damage (7). Ionizing radiation sensitizes cells to topo-1 inhibitors by slowing their progression through S-phase, thus, increasing the number of cells in S-phase (4). The most optimal effects *in vivo* have been seen when the topo-1 inhibitor is given shortly before the irradiation (5), or concurrently with continuous application (8, 9).

While external beam irradiation of loco-regional disease is possible in a disease like primary non-small cell lung cancer, this is a less feasible approach with respect to metastatic breast cancer which may be more widely disseminated. Radioimmunoconjugates provide a vehicle for targeting therapeutic doses of radiation to widely dispersed tumor throughout the body. Similarly, the above principles of synergy will apply to radiation delivered by this method as well as by external beam, but with improved therapeutic index.

As previously reported, we have demonstrated the feasibility of administering topotecan as a continuous infusion in the mouse model. We also demonstrated an antitumor effect. As described below we have continued with our experiments using continuous infusion topotecan and radioimmunoconjugate. The data generated thus far support the use of combination therapy for future clinical trials.

**Body**

Over the last year, we have completed a phase I study using $^{111}$In-MX-DTPA huBrE-3 in patients with advanced breast cancer (previously submitted to you.). To date, 87 patients have been referred for screening of tissue for BrE-3. Tissue blocks were available in 58. Of these, 35 patients' tissue demonstrated staining of > 25% of the cells in the tumor. Fourteen of our patients have had only fine needle aspiration (FNA) which we previously were unable to stain. With a new fixing technique, we are currently able to stain FNA specimens and in two patients we have obtained positive results.

We studied 7 patients on this protocol. They each received 2 mg of MX-DTPA huBrE-3 labeled with about 5mCi of Indium $^{111}$ plus 48 mg of nonconjugated BrE-3 intravenously over one hour. The antibody infusions were well tolerated. No allergic or toxic side effects were observed. One patient complained of a transient strange taste in her mouth. One patient developed grade 3 thrombocytopenia at 9 days after infusion of antibody. She was concurrently receiving external beam radiation to the spine and had extensive involvement of the bone marrow with metastatic carcinoma documented by bone marrow biopsy. Serologic analysis revealed no evidence of immunologic platelet destruction and it was felt that the thrombocytopenia was predominantly secondary to the combination of poor bone marrow reserve and external beam radiation and unlikely to be related to toxicity from the $^{111}$In MX-DTPA huBrE-3. Patients underwent serial whole body counting, gamma camera imaging, plasma and urine sampling over one week in order to assess pharmacokinetics, radiation dose, tumor localization and pharmacokinetics. We imaged 76% of known bone, liver, and lung lesions and identified two sites previously unsuspected (lymph node, bone). Blood pharmacokinetics show a longer half-life for the humanized antibody than for the murine. In six patients the $T1/2_{alpha}$ for the humanized antibody averaged $106.5 \pm 8.5$ hours and the $T1/2_{beta}$ averaged $114.2 \pm 39.2$ hours. Radiation dose estimates for (using standard MIRD formalism) have been made for normal organ and tumor. Dose estimates to tumors averaged $82 \pm 22$ rads/mCi administered for $^{90}$Y-MX-DTPA huBrE-

3 with average marrow dose estimated at 5±3 rads/mCi administered (see appendix tables 1 and 2). Immunogenicity has now been studied out to 3 months in 3 of our patients. Qualitative analysis of serum incubated with either radioiodinated huBrE-3 or Indium-111 labeled hu BrE-3 demonstrates that compared to baseline serum, there are trace amounts of antibody-antibody formation at 5 weeks and 3 months after antibody infusion. Since these anti-humanized antibodies react equally with the murine BrE-3, we believe that "HAHA" represents an idiotypic response.

Thus far, the results suggest that it is possible to administer therapeutic doses of radioimmunoconjugates to patients. The relatively low immunogenicity may allow for repeated administration. We have just closed the radioimaging study and have had recent IRB approval of a phase I radiotherapeutic study using dose fractionated Y-90 MX-DTPA huBrE-3 in patients with advanced breast cancer (protocol in appendix). We are in the process of screening patients who are potential candidates for this study. We ultimately plan to proceed with a combination phase I trial of radioimmunotherapy and topoisomerase I inhibitor therapy based on the promising results of our preclinical work previously described and discussed below.

Over the past year we expanded upon our initial observations demonstrating significant anti-tumor efficacy of combination continuous infusion topotecan and Y-90 MX-DTPA BrE-3 in the mouse model. As previously described, we conducted a series of experiments in the athymic female nude mouse model implanted with the human transplantable breast tumor line MX-1 in the left flank. In the last experiment previously reported, on day 21 after implantation of MX-1, mice were randomized into four treatment groups: Control (no treatment), BrE-3 (i.p. 50 ug of murine MX-DTPA BrE-3 labeled with 200uCi of $^{90}$Yttrium), topotecan (1mg/m2 for 14 days via s.c. Alzet pump), and combination (BrE-3 and topotecan). Body weights and tumor weights were measured every 3-4 days. As noted in Figure 1, the control mice all died by day 69 after tumor implantation. In the groups treated with BrE-3 or topotecan alone, the mice had reduced tumor growth for about 50 days post treatment but then the tumor grew to sizes comparable to the untreated tumor bearing mice. The mice that received the combination therapy had a substantial decrease in tumor cell growth that resulted in compete tumor regression in 10 of 13 mice. At sacrifice 120 days after treatment, none of the surviving 10 mice had any sign of recurrent tumor.

Our next experiment was performed in order to determine whether the observed tumor response is primarily due to the specificity of the 90Y-MX-DTPA-BrE-3 or to the systemic circulating level of radioactivity given to the animal. A non-specific, isotype matched-matched monoclonal antibody(MOPC) was used for the combined therapy. In addition, the humanized BrE-3 was substituted for the murine antibody as the humanized BrE-3 is used in clinical trial as previously described. Athymic female nude Swiss NIH mice were implanted with human mammary carcinoma (MX-1). On day 21, mice were randomized into one of 5 groups as follows:

| Group # | hu-90-Y-BrE-3 | 90Y-MOPC | Topo (1mg/m2 x14days) |
|---|---|---|---|
| 1 (n=6) | - | - | - |
| 2 (n=6) | + | - | - |
| 3 (n=6) | - | + | - |
| 4 (n=6) | + | - | + |
| 5 (n=6) | - | + | + |

As shown in Figure 2, their was no significant tumor inhibition noted in group #3 and their was no difference in survival between the control group and group #3. The synergistic effect noted with the combined 90Y-MX-DTPA BrE-3 and Topo was not observed in the combined MOPC and topotecan group. Only a transient inhibition of tumor growth was noted in group #5 which was similar in effect and survival to the group treated with BrE-3 alone. The huBrE-3 in combination with topotecan demonstrated the same synergism as with the murine antibody.

Our next experiment was performed to evaluate treatment efficacy and morbidity in animals treated with a single dose of 200uCi 90Y labeled MX-DTPA BrE-3 compared to two fractionated doses of 90Y labeled MX-DTPA BrE-3 in combination with topotecan. On day 21 post tumor implantation, mice bearing MX-1 tumors were injected either with a single dose of 200uCi 90Y labeled with MX-DTPA BrE-3 or with 2 weekly injections of 125uCi 90Y labeled with MX-DTPA BrE-3. Mice in each group were then randomized to receive topotecan (1mg/kg for 14 days) via Alzet pump as follows:

| Group # | 200uCI BrE-3 + Topo | 125uCi BrE-3x2 | 125uCiBrE-3 x2+Topo |
|---|---|---|---|
| 1(n=6) | - | - | - |
| 2(n=6) | + | - | - |
| 3(n=6) | - | + | - |
| 4(n=6) | - | - | + |

As shown in Figure 3, both combination groups #2 and #4 had dramatically reduced tumor growth which were of the same magnitude in the fractionated 90Y labeled dosing as in the single dose.

In order to help elucidate the cell-damage mechanism of the combined therapy, several *in vitro* experiments were performed to determine the effect of topotecan, 90Y labeled BrE-3, and the combination on cell proliferation. Two tumor cell lines were utilized for these *in vitro* studies, the MDA-MB 157 (BrE-3 positive) and the MDA-MB 435 (BrE-3 negative) human mammary carcinoma's. Colorimetric (MTT) assay is used to determine cell survival and proliferation. MTT(3-(4,5-dimethylthiazol-2-yi)-2,5 diphenyl tetrasolium bromide) is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria. In this experiment, $1x10^4$ cells are plated in 96 wells plate overnight in DMEM media with

10% FBS. 90Y-BrE-3 (5ng to 500ng Ab proteins) are added to half of the wells and incubated for 1 hour. Cells were washed three times to remove all the unbound 90Y-BrE-3 antibodies. Escalating concentrations of topotecan (0uM to 10uM) were then added to the appropriate wells. Cells incubated in media alone served as controls. Plates were incubated for four days and MTT were then added to the wells and allowed to incubate for an additional four hours. Cells were lysed with HCL/isopropanolol and absorbance measured on an ELISA plate reader with a test wavelength of 570 nm and a reference wavelength of 630 nm. BrE-3 binding affinity is ten-fold higher in the MDA-MB 157 than in MDA-MB435. In both cell lines, topotecan exerts a dose escalating reduction of cell proliferation rate (see figure 4). However, in the presence of 90-Y monoclonal Abs, only the MDA-MB 157 cell line which retained 90-Y BrE-3 exhibited a further decrease in cell proliferation rate (data not shown).

In addition, an *in vitro* assay was established with the MDA-MB 157 cell lines and the MDA-MB 435 cell lines described above. Similar to the last experiment, $1x10^4$ cells are plated in 96 wells overnight in DMEM media with 10% FBS. 90Y BrE-3 (5 ng to 500 ng Ab proteins) were added to half of the wells and incubated for 1 hour. Cells were then washed three times to remove all the unbound 90Y BrE-3 antibodies. Escalating concentrations of topotecan (0uM to 10uM) were then added to the appropriate wells. At the end of 3 days incubation, cells were lysed and supernatant collected for determining rate of apoptosis. The assay used to measure the degree of apoptosis (Cell Death Detection ELISA, Boehringer Mannheim) is based on the quantitative sandwich-enzyme-immunoassay-principle using a mouse monoclonal antibodies directed against DNA and histones, respectively. This allows the specific determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Briefly, anti-histone antibody is fixed absorptively on the wall of the microtiter plate. Supernatant which contained the cytoplasmic fraction of cells were added to the wells and allowed to incubate at room temperature for 90 minutes. After several washings, anti-DNA-peroxidase conjugating solution was added to the wells for an additional 90 minutes incubation at room temperature. Substrate solution was added for photometric analysis and absorbance was measured on an ELISA plate read with a test wavelength of 405nm and a reference wavelength of 490nm. As shown in figures 5 and 6, topotecan is able to induce and increase of apoptosis in a dose escalating manner for both MDA-MB157 and MDA-MB435 cell lines. No additive increase is seen in MDA-MB435 cells treated with 90-Y BrE-3 monoclonal antibody. For the MDA-MB157 cells, however, their is an increased rate of apoptosis in the presence of BrE-3 alone and an additive effect is demonstrated when both topotecan and BrE-3 are administered in combination.

## Conclusion

In conclusion, over the last year we have completed a radioimaging study of $^{111}$In-Mx-DTPA-huBrE-3 in patients with metastatic breast cancer. We are in the process of preparing a manuscript for publication. The results suggest that a therapeutic trial of 90-Y-Mx-DTPA huBrE-3 is feasible and repeated doses can be administered. We have recently opened a therapeutic phase I study described above. In addition we have continued to perform our experiments in the mouse model with combination

radioimmunotherapy and continuous infusion topotecan. As previously noted, the experiments demonstrated that topotecan enhances the therapeutic index of radioimmunotherapy against human mammary carcinoma. Substituting a nonspecific MOPC antibody for BrE-3 did not result in an anti-tumor response indicating that the specific binding of the BrE-3 antibody to the tumor is necessary. In addition, substituting murine BrE-3 for humanized BrE-3 resulted in similar antitumor efficacy. Using a fractionated dose of BrE-3 given two times resulted in a similar anti-tumor effect to one dose of BrE-3. Our *in vitro* studies demonstrated a decrease in cell proliferation rate and an increase in the apoptotic rate in the cells treated with topotecan. The MDA-MB 157 cells which are BrE-3 +, demonstrated an additive effect on the apoptotic rate when treated with a combination of BrE-3 and topotecan. The data generated thus far continue to support the rationale for using this combined modality therapy in women with advanced breast cancer. In the upcoming year we plan to complete our phase I study of fractionated 90Y-BrE-3 and to initiate a phase I study of combined radioimmunotherapy and continuous infusion topotecan in women with advanced breast cancer. In our preclinical experiments, we will investigate other chemotherapeutic agents such as paclitaxel and gemcitabine in combination with radioimmunotherapy. If this or other agents reveal synergistic activity we will pursue studying these combinations in patients.

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APPENDIX

**Dose estimates based on biodistribution of In-111 MX-DTPA huBrE-3 (rads/mCi)**

Table 1

**Indium-111 (rads/mCi)**

| | 01HBR | 02HBR | 03HBR | 04HBR | 05HBR | average | S.D. |
|---|---|---|---|---|---|---|---|
| kidneys | 1.99 | 2.73 | 1.68 | 3.22 | 2.41 | 2.41 | 0.61 |
| liver | 5.91 | 2.71 | 3.03 | 2.17 | 2.03 | 3.17 | 1.58 |
| lung | 1.01 | 0.81 | 0.83 | 1.34 | 1.33 | 1.06 | 0.26 |
| ovaries | 0.51 | 0.46 | 0.41 | 0.52 | 0.56 | 0.49 | 0.06 |
| red marrow* | 0.5 | 0.45 | 0.51 | 0.66 | 2.09 | 0.84 | 0.70 |
| spleen | 3.46 | 2.08 | 1.69 | 1.9 | 1.74 | 2.17 | 0.73 |
| urinary bladder | 0.75 | 0.37 | 0.34 | 0.42 | 0.38 | 0.45 | 0.17 |
| whole body | 0.621 | 0.48 | 0.45 | 0.52 | 0.54 | 0.52 | 0.07 |

Table 2

**Yttrium-90 (rads/mCi)**

| | 01HBR | 02HBR | 03HBR | 04HBR | 05HBR | average | S.D. |
|---|---|---|---|---|---|---|---|
| kidneys | 14.9 | 27.6 | 14.6 | 33.8 | 23 | 22.78 | 8.27 |
| liver | 46.9† | 19.3† | 22.2† | 14.7 | 13.4 | 23.30 | 13.66 |
| lung | 5.85 | 5.99 | 6.39 | 13.7†† | 13.2 | 9.03 | 4.05 |
| ovaries | 1.5 | 1.45 | 1.27 | 1.62 | 1.26 | 1.42 | 0.15 |
| red marrow* | 2.02 | 1.9 | 3.89 | 5.69 | 9.85 | 4.67 | 3.29 |
| spleen | 37.4 | 19.5 | 16.10 | 16.6 | 15.2 | 20.96 | 9.33 |
| urinary bladder | 1.81 | 1.07 | 1.09 | 1.31 | 0.94 | 1.24 | 0.34 |
| whole body | 2.82 | 2.27 | 2.01 | 2.35 | 2.38 | 2.37 | 0.29 |
| tumors | 101.35 | 89.63 | 92.8/81.4 | ** | 43.7 | 81.78 | 22.45 |

*based on blood

†Patients 2 and 3 had liver metastases. Patient 1 manifested diffuse metastases on CT within 3 months.

†† multiple lung metastases

**No measurable tumors visualized on planar images. Dosimetry could not be calculated.

Figure 1

# Effect of Combined Treatment of Topotecan & BrE-3 on Breast Cancer Xenografts

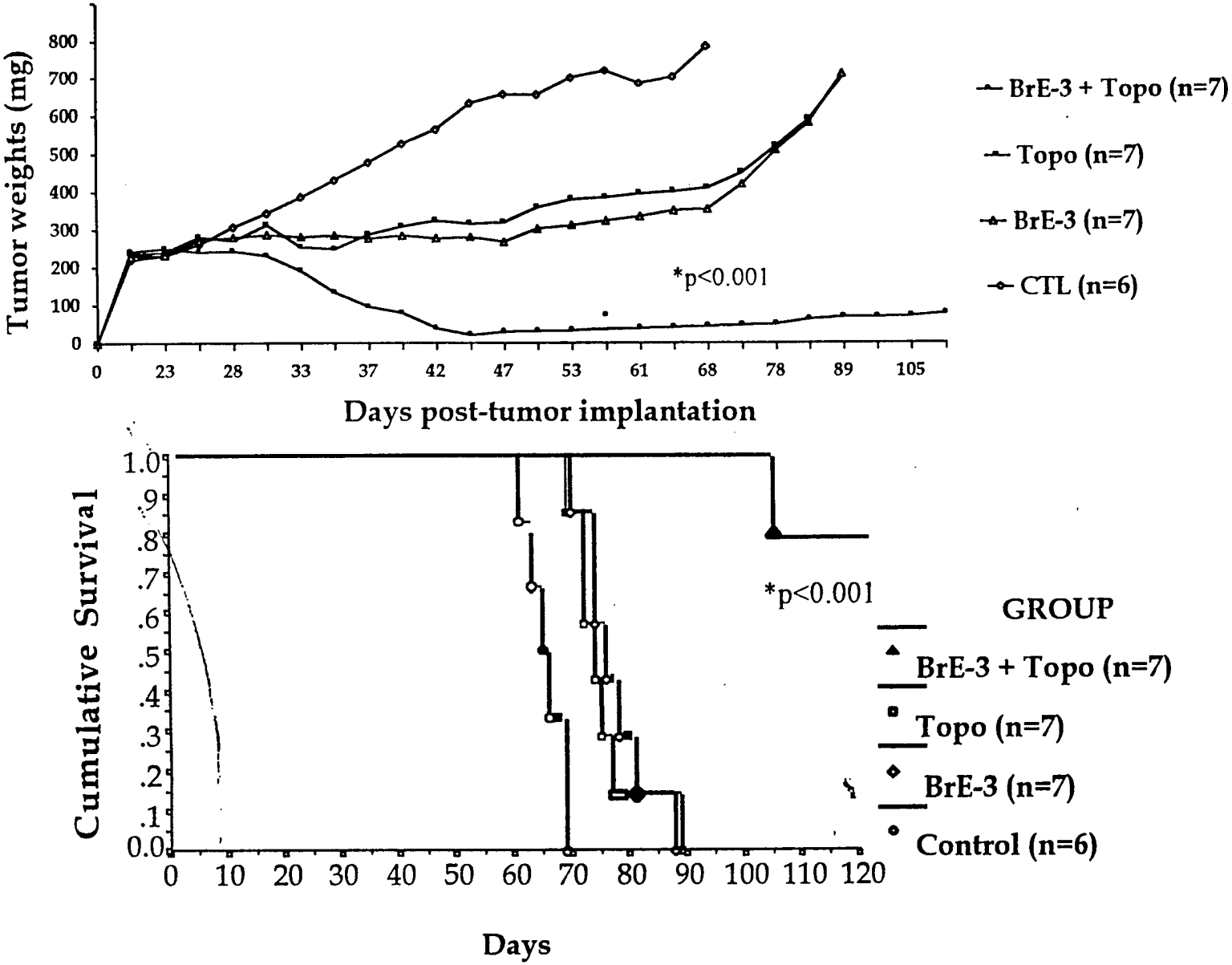
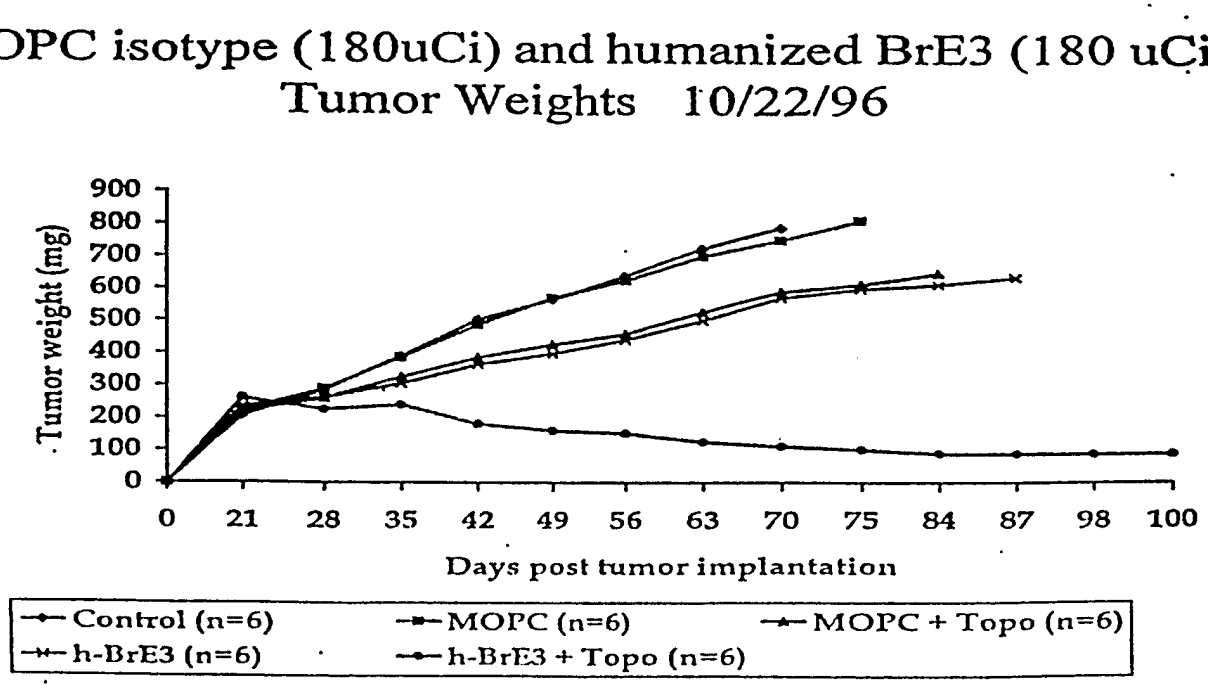


Figure 2




Effects of combination treatment with humanized BrE3 and non-specific radiolabeled antibody (MOPC). The MOPC alone was comparable to the not treatment animal group, while the combination MOPC/topotecan was comparable to the topotecan treatmetn group alone. The combination HuBrE-3 topotecan showed the same syngerestic activity observed with the murine BrE-3 antibody.

Figure 3: Effect of Fractionated BrE-3 on Breast Tumor Xenografts

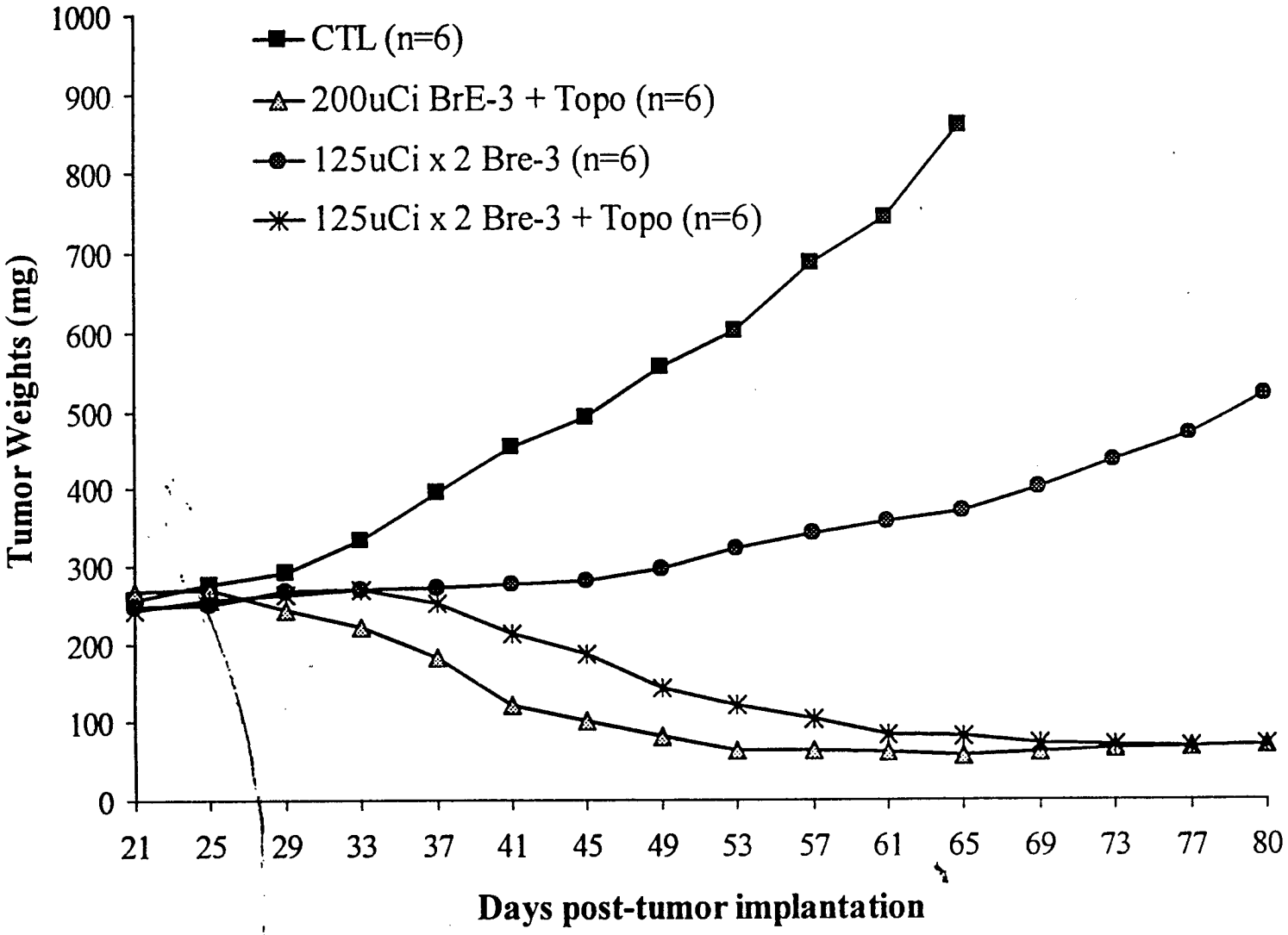
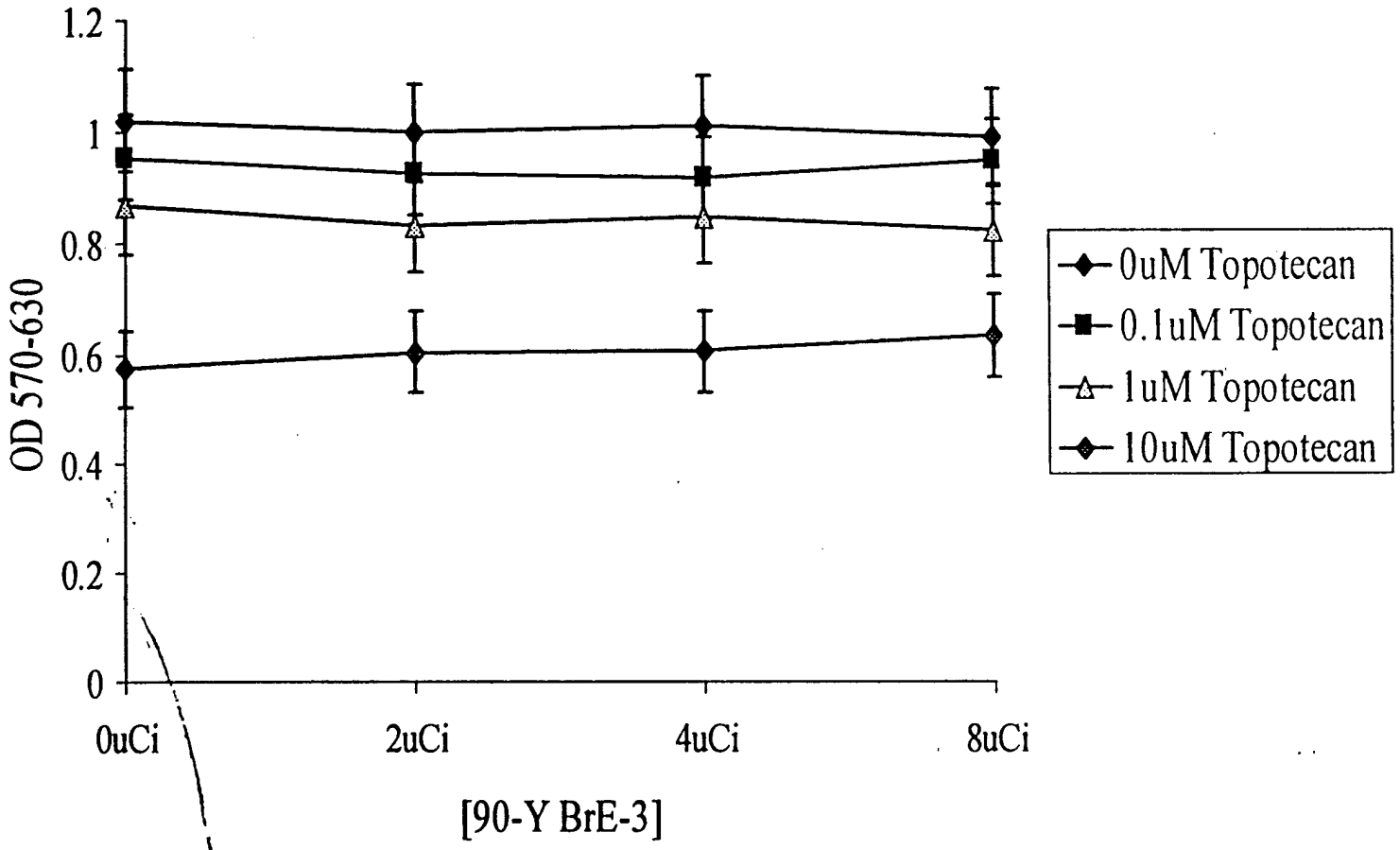


Figure 4

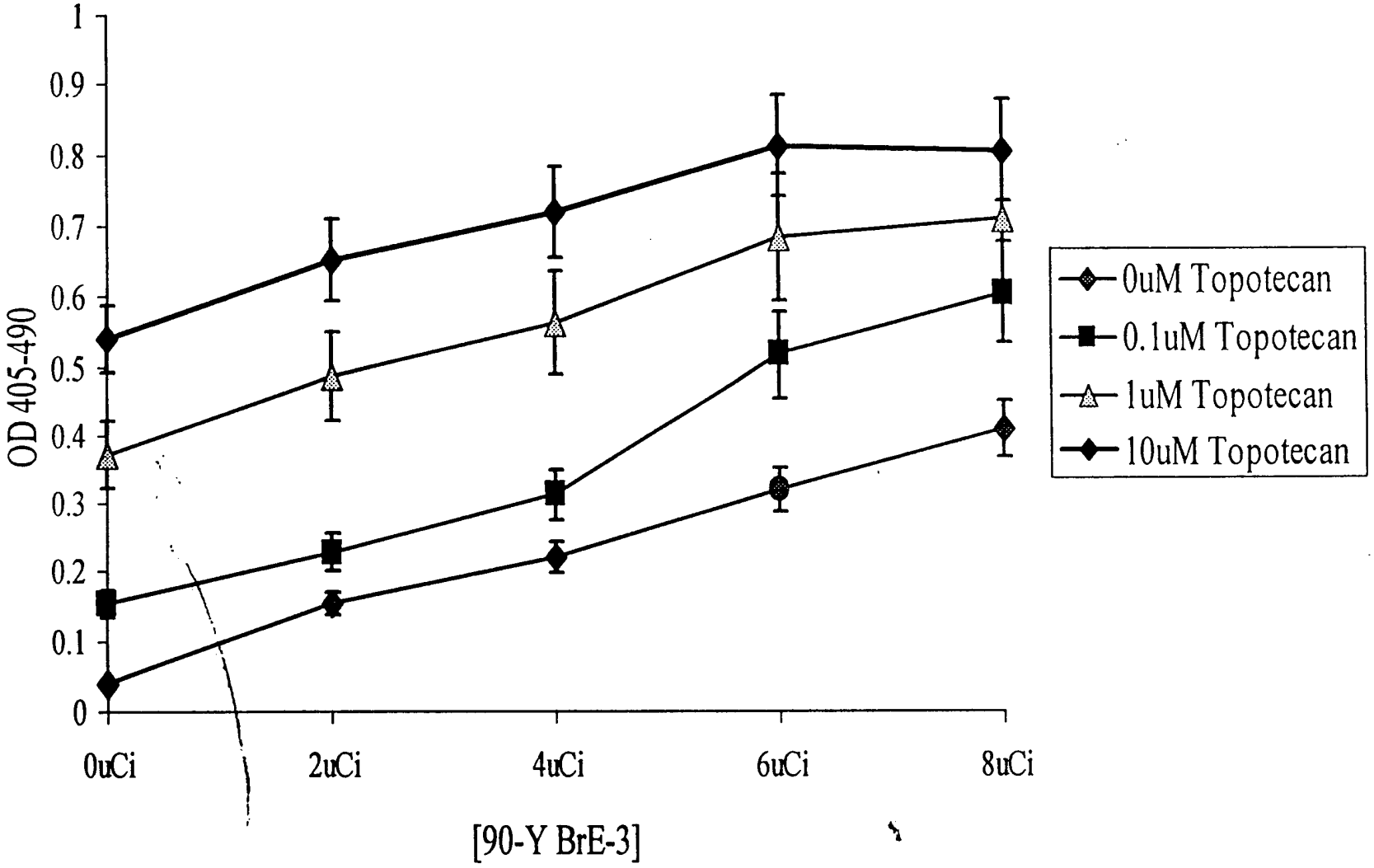



MTT Cell Proliferation: MDA-MB 435 (BrE-3 negative)

Figure 5


0uM Topotecan
0.1uM Topotecan
1uM Topotecan
10uM Topotecan
OD 405-490
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0uCi
2uCi
4uCi
6uCi
8uCi
[90-Y BrE-3]
Apoptosis: MDA-MB 435 (BrE-3 negative)

Figure 6




Apoptosis: MDA-MB 157 BrE-3 positive

**Protocol: A Phase I study of the Toxicity and Dosimetry of a Humanized Breast-Directed Monoclonal Antibody (BrE-3) radiolabeled with $^{111}$Indium ($^{111}$In) and $^{90}$Yttrium ($^{90}$Y)**

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C.1 Overview

It is the overall aim of this proposal to use humanized antibodies directed against breast epithelial antigens and an improved understanding of the epitopes targeted by these antibodies to develop effective radioimmunotherapy strategies in patients with metastatic and/or recurrent breast cancer. Two of the most significant obstacles to effective radioimmunotherapy have been the dose limiting radiation toxicity to bone marrow and the immunogenicity of the immunoconjugates used. The work proposed here will be based on a newly gained understanding of the in vivo behavior of humanized radioimmunoconjugates in an attempt to overcome these obstacles. This protocol will capitalize on the expected reduced immunogenicity of the humanized radioimmunoconjugates and employ a dose fractionation approach to attempt to increase the therapeutic index of these radioimmunoconjugates. Our initial efforts will focus on determination of the pharmacokinetics, biodistribution and toxicity of $^{90}$Y- labeled huBrE-3 antibody, which based on our recent experience with the murine construct, is expected to show promising in vivo localization in tumors of patients with metastatic breast carcinoma. Clinical Phase I trials will be implemented to evaluate $^{90}$Y BrE-3 in a dose fractionation regimen. Although it is expected that dose fractionation may mitigate the marrow toxicity, it is expected that marrow toxicity secondary to radiation exposure will be remain the dose-limiting toxicity. Therefore,we will explore the respective radioisotope content of the marrow and bone as well as the effect on hematopoietic precursors. The NYU Cancer Antibody Trials Group is fully experienced with all aspects of the work proposed, both in preclinical and clinical systems. Extensive, research oriented, clinical services with a long history of performing complex therapeutic protocols, assures high patient accrual rates and careful collection of the necessary data. These studies will be carried out under the following specific aims:

These studies are focused on the development of an effective treatment for breast cancer using radioimmunoconjugates. Because of its very favorable specificity, the results of preclinical studies demonstrating significant therapeutic activity, and the clinical data which suggests good tumor localization and promising dosimetry, we will use antibody BrE-3.

The timeline (see below) outlines our plans for the project period according to the following specific aims:

Specific Aim 1: To determine the toxicity profile and maximum tolerated dose of fractionated radioimmunotherapy using $^{90}$Y- labeled huBrE-3 antibody in a Phase I trial in patients with advanced breast carcinoma.

Specific Aim 2: To examine the biodistribution and pharmacokinetics of both $^{111}$In labeled BrE-3 and $^{90}$Y labeled BrE-3 and the marrow toxicity of the $^{90}$Y labeled immunoconjugate.

a) To measure the radiotoxic effect of intravenously administered radioimmunoconjugates directly using hematopoietic progenitor assays.

b) To correlate the marrow and bone localization of $^{111}$In with that of $^{90}$Y after coadministration of immunoconjugates labeled with each of these radioisotopes.

c)To specifically examine the radiation dose to the marrow estimated for an $^{90}$Y immunoconjugate using the biodistribution of $^{111}$In BrE-3 in relation

to the actual effect on marrow as assessed by hematopoietic progenitor assays and peripheral blood counts.

Specific Aim 3: To determine the safety and efficacy of dose fractionated $^{90}Y$- labeled huBrE-3 antibody in patients with advanced breast cancer.

This study will be a Phase I trial of dose fractionated $^{90}Y$-humanized BrE-3. This isotope has been selected because of its pure ß emissions and relatively good tumor retention. A dose escalation schedule for the therapeutic isotope will be followed to evaluate maximum tolerated dose. Because $^{90}Y$ cannot presently be imaged satisfactorily, we will coinfuse $^{111}In$-BrE-3 at a constant dose with the first administration. The designated dose of $^{90}Y$ will be administered on Day 1. Then using blood levels and whole body counting to determine clearance of $^{90}Y$, a second dose ( and possibly third dose) will be administered to return to the initial level of radioactivity. Clinical monitoring studies will be carried out to examine the pharmacokinetics and distributions of the $^{90}Y$ and $^{111}In$ radioimmunoconjugates. We will assess distribution after the first administration with planar and SPECT imaging, and after each administration determine pharmacologic parameters using serial blood and urine sampling, and tumor biopsies, where possible. We will perform marrow aspirations at the end of the first cycle. We will determine amounts in serum of non-antibody bound radionuclide, degradation products, and intact antibody, for both isotopes, to assess stability and metabolism. Based on data available to date, we anticipate that the behavior of the two conjugates will be similar, with differences (e.g., bone uptake) being predictable. We anticipate that dose-limiting toxicity for this radioconjugate will be myelosuppression.
The studies proposed are based on the use of humanized BrE-3, presently the most promising of the human milk fat globule membrane antigen monoclonal antibodies because of its high expression on breast tumor cells, low levels of available antigen shed into the circulation, preclinical studies demonstrating therapeutic efficacy in model systems, and preliminary Phase I localization and pharmacokinetic data which show favorable blood pharmacokinetics and good tumor localization.

C.2 Schema

| Number of | Dose huBrE-3(mg) | | Radioactivity. | |
|---|---|---|---|---|
| Patients | Unlabeled | Radiolabeled * | $^{111}In$ | $^{90}Y$ |
| 3 | 38 | 2 /10 | 5 mCi | 3.0 mCi/ $m^2$ X 2 |
| 3 | 38 | 2/10 | 5 mCi | 4.5 mCi/ $m^2$ X 2 |
| 3 | 38 | 2/10 | 5 mCi | 6.0 mCi/ $m^2$ X2 |
| 3 | 38 | 2/10 | 5 mCi | 7.5 mCi/ $m^2$ X2 |

* 2 mg of $^{111}In$ huBrE-3-benzyl methyl DTPA and 10 mg of $^{90}Y$ BrE-3-benzyl methyl DTPA

C.3 Patient eligibility

C.3.1 Patients must have histologically confirmed, metastatic or recurrent breast carcinoma which expresses BrE-3 antigen.

C.3.2 Patients must have measurable or evaluable disease.

C.3.3 Karnofsky performance status of $\geq$ 70 % ( ECOG 0,1,2)

C.3.4 Patients must have adequate organ function as defined by:

C.3.4.1 Neutrophil count $\geq$ 2500 and platelet count > 100,000

C.3.4.2 Bilirubin < 2.0.

C.3.4.3 Creatinine $\leq$ 2.0 or creatinine clearance $\geq$ 40 ml/ minute.

C.3.4.4 Normal chest radiograph or pO2 $\geq$ 80mm Hg on room air and involvement by tumor of $\leq$25% of pulmonary parenchyma as assessed by CT.

C.3.5 No evidence of active infection which requires antibiotic therapy.

C.3.6 Patients entering this study must be 3 weeks post chemotherapy or radiation therapy and have recovered fully from the toxic effects. Patients may not be on concurrent chemotherapy or radiation therapy. Patients must have failed at least 1 prior standard chemotherapy regimen.

C.3.7 Patients with no prior exposure to monoclonal antibodies or serum which is non-reactive to huBrE-3.

C.3.8 Patients must be at least 18 years of age.

C.3.9 Women of child-bearing potential must have a negative pregnancy test.

C.3.10 Evidence of the BrE-3 antigen on immunohistochemistry using BrE-3 antibody in at least 50% of the tumor cells.

C.3.11 Serum BrE-3 antigen level $\leq$ 10µg/ml or a total of 25 mg/total plasma volume

C.4 Exclusion criteria

C.4.1 Patients with evidence of an active second malignancy are not eligible. Patients with a history of a second malignancy, but no evidence of active disease related to this malignancy, may be considered eligible at the discretion of the investigator.

C.4.2 Clinically significant cardiac disease (New York Heart Association Class III/IV)

C.4.3 Serious infection requiring treatment with antibiotics or other serious concurrent illness.

C.4.4 Concurrent steroid therapy.

C.4.5 Pregnancy or lactation

C.4.6 Survival expectancy less than 12 weeks

C.4.7 Active CNS tumor involvement precludes eligibility . This includes spinal cord involvement.

C.4.8 Evidence of extensive skeletal metastases as assessed by bone scintigraphy ;> 25% of the axial skeleton. Bilateral pelvic (sacroiliac) metastases will exclude the patient from eligibility.

C.4.9 Prior exposure to monoclonal antibodies and evidence of anti-huBrE-3 antibody.

C.5 Drug Information

C.5.1 The monoclonal antibody will be provided by the Cancer Research Fund of Contra Costa, in a final concentration of about 6.9 mg/ml mg/ml. It will be prepared from tissue culture supernatants in hollow fiber bioreactors. It is purified using Protein A and Q Sepharose columns. Purified product will be sterilized through a O.2 u filter. General safety, sterility, pyrogenicity, polynucleotides, mycoplasma, and adventitious virus contamination were tested in accordance with a Notice of Claimed Investigational Exemption for a New Drug (IND) (Office of Biologics, U.S. Food and Drug Administration). All clinical studies will be performed with material prepared under an IND.

C.5.2 The methyl benzyl DTPA conjugated BrE-3 for radiolabeling will be provided by Cancer Research Fund of Contra Costa (CRF) in a concentration of 2 mg/ml. Sterile, low pyrogenic solutions of acetate buffer and sodium/calcium EDTA in saline will be prepared at NYU. The Indium-111( sterile, low pyrogenic) will be purchased INS.IPA from Amersham, Inc. The Yttrium-90 (sterile, low pyrogenic) will be purchased from Nordion, Inc.

C.6 Immunoconjugation

Radiolabeling of antibody will be performed under the supervision of Dr. Elissa Kramer in the laboratory located in the Division of Nuclear Medicine of Tisch Hospital.

C.6.1 Chelate

A Methyl benzyl DTPA huBrE-3 conjugate will be provided by CRF. The purity of benzyl DTPA huBrE-3 has been demonstrated by reduced and non-reduced SDS-PAGE stained with coomassie brilliant blue and HPLC sizing column ( Indium-111 labeled). The average number of DTPA's per BrE-3 molecule will be approximately 1.1 as estimated using cold competing Indium.

C.6.2 Labeling with $^{111}In$

Methyl benzyl DTPA BrE-3 will be radiolabeled under an IND protocol by mixing 2 mg of the antibody chelate with 5-10 mCi of acetate buffered $^{111}In\ Cl_3$ for 20 minutes at room temperature. The reaction mixture is then challenged with a 5mM sodium/calcium EDTA solution.

C.6.2 Labeling with $^{90}Y$

In addition, Methyl benzyl DTPA BrE-3 will be radiolabeled under an IND protocol by mixing 10 mg of the antibody chelate with the requisite mCi of acetate buffered $^{90}Y$ chloride for 20 minutes at room temperature.

The labeled preparations ( both $^{111}In$ and $^{90}Y$) will be purified by gel filtration on a previously autoclaved 10 cm Sephadex G-80 columns. The fractions corresponding to the immunoglobulin fraction will be pooled and diluted into 0.9% saline solution containing 1% human serum albumin (total volume of 18 ml)

C.6.3 Quality control

A 0.1 ml of the product will be withdrawn for radiochemical purity and immunoreactivity determinations.

Three milliliters of the final products will be withdrawn for USP sterility testing and for pyrogen testing.

The radiochemical purity of the labeled antibody will be determined by ITLC chromatography using O.9% saline/5 mM EDTA eluant. The radiochemical purity of the final product will be >90% for patient administration. Criteria for radiochemical purity and apyrogenicity will be met before the labeled antibody can be administered to the patient. Immunoreactivity of this preparation will be determined using an antigen bead radioimmunogen procedure by applying infinite antigen excess (17). The minimum acceptable immunoreactivity will be 60%. The chromagenic assay test for pyrogens will be within acceptable limits for patient dosing. The sterility test will be performed according to the guidelines prescribed in the United States Pharmacopeia.

C.6.4 Preparation for infusion

Any unlabeled antibody to be administered will be mixed with the radiolabeled antibody in a total volume of 250 ml of 0.9% saline solution containing 1% human serum albumin.

C.6.5 Storage

The radiolabeled MoAb BrE-3 will be stored at 2° - 8° C in a shielded environment while the quality control procedures are in progress. Upon receiving a satisfactory report about the radiochemical purity and an acceptable level of pyrogens in the labeled MoAb, it will be made available in the Department of Nuclear Medicine for administration to the patient.

C.6.6 Drug Accountability

A record of receipt, usage and disposition of huBrE-3 and methyl benzyl DTPA huBrE-3 will be kept in the Division of Nuclear Medicine. A record of receipt, usage and disposition of all radionuclides related to this protocol will be kept in the Division of Nuclear Medicine.

C.7 Study Design

All patients will undergo an evaluation for the purposes of determining and measuring other sites of evaluable disease. This includes a thorough history and physical examination, blood counts and chemistry surveys, routine chest x-ray and electrocardiogram, and urinalysis, computerized tomograms (CT scans) of appropriate areas, and bone scan. A baseline bone marrow aspiration will be obtained. In addition, assays for free circulating antigen which is recognized by the therapeutic antibody will be performed, as well as assays for human antibodies against huBrE-3 antibody. This will be a study of escalating amounts and serial doses of $^{90}Y$ radioactivity labeled to huBrE-3 antibody. Cohorts of 3 patients at each level will be studied according to the schema detailed below. Pharmacokinetics and serial scanning to determine the biodistribution and radiation dosimetry will be performed. Toxicity and response will be assessed using standard criteria (detailed below). If a response is demonstrated to the first administration in a patient, repeat administration at the same dose level will be considered. At MTD a Phase II study will be performed incorporating the initial patients studied at MTD in the course of dose escalation. A two part study will be performed so that the trial can be terminated early if the $^{90}Y$ immunoconjugate is not active in these patients.

C.7.1 Informed consent

The protocol will be discussed in detail with each patient prior to enrollment and written informed consent according to the guidelines of the Institutional Board of Research Associates of New York University will be obtained.

C.7.2 Antibody administration

Patients accepted on this protocol will receive an infusion consisting of a tracer dose of $^{111}In$ labeled antibody together with a therapeutic dose of $^{90}Y$ labeled antibody (Day 1). The $^{111}In$ labeled antibody will be used to establish biodistribution patterns of this antibody, the degree of tumor localization, and dosimetric parameters and will be compared with that of the $^{90}Y$ labeled BrE-3 and other forms of $^{90}Y$ in blood, urine, and when possible, bone marrow biopsies and tumor biopsies. The $^{90}Y$ infusion will be repeated after 1 week (Day 8) and with further dose escalation, at Day 15 as well.

For this Phase I study all patients will be out-patients at either Tisch Hospital or Bellevue Hospital Center unless anticipated external radiation exposure rates exceed the radiation safety guidelines. They will be monitored in the Division of Nuclear Medicine in the hospital up to 2 hours post infusion. All patients will receive coinfusions of 5 mCi of $^{111}In$ BrE-3-methyl benzyl DTPA and escalating doses of $^{90}Y$ BrE-3-methyl benzyl DTPA as detailed in the schema followed by serial gamma camera images during the first week through day 8. Blood clearance and imaging for organ uptake studies will also be performed on each patient. Based on our recent experience in the Phase I trial of In-111 -murine and humanized BrE-3 monoclonal antibodies, we have determined that a 50 mg dose of antibody may be administered without undue toxicity and that this will permit delivery of radiolabeled antibody to tumor as long as circulating antigen levels do not exceed 10 μg/ml.

C.7.2.1 Dose escalation

This will be a dose escalation study to determine the MTD of $^{90}Y$ methyl benzyl DTPA BrE-3 antibody with cohorts of 3 patients entered initially at each of the following dose levels:

| | Radioactivity |
|---|---|
| $^{111}In$ | $^{90}Y$ |
| 5 mCi (1st admin. only) | 3.0 mCi/ $m^2$ weekly X 2 |
| 5 mCi (1st admin. only) | 4.5 mCi/ $m^2$ weekly X 2 |
| 5 mCi (1st admin. only) | 6.0 mCi/ $m^2$ weekly X 2 |
| 5 mCi (1st admin. only) | 7.5 mCi/ $m^2$ weekly X 2 |

At MTD a total of up to 30 evaluable patients will be studied to determine tumor response rate and activity of this therapy in advanced breast cancer.

C.7.2.2 Criteria for dose escalation (Figure 1)

Dosages will not be escalated over successive treatment courses for individual patients. All of the initial three patients at a given dose level will be observed for four weeks before additional patients are added. All patients at each dose level will be observed for a period of four weeks after the last radioimmunoconjugate administration before any patients are enrolled at the next higher dose level.

It is estimated that approximately 18-24 patients per year will be enrolled using this schema.

C.7.2.2.1 If no grade III( non-hematologic) or grade IV toxicity ( by common toxicity criteria, see Appendix 1) is observed among the first three patients at a given dose level, the dose will be increased for the successive group of three patients.

C.7.2.2.2 If at least one patient among the first three patients at a given dose level experiences Grade III( non-hematologic) or IV toxicity (hematologic and < 1 week in duration), an additional three patients will be treated at that level.

C.7.2.2.3 If one patient experiences Grade IV (nonhematologic) toxicity or if one patient experiences Grade IV hematologic toxicity of ≥ 1 week duration, no more patients will be treated at that dose level and the maximum tolerated dose will have been exceeded. Three more patients will be treated at the next lower dose level, if necessary, to reach a total of 6 patients at that lower dose level.

C.7.2.2.4 Of the 6 patients at a given dose level, if only one patient experiences dose limiting toxicity (Grade III nonhematologic or Grade IV hematologic lasting < 1 week), the dose will be increased for the successive group of three patients.

C.7.2.2.5 Of the 6 patients at a given dose level, if 2 patients experience Grade IV(hematologic and < 1 week in duration) toxicity, or if three patients experience Grade III nonhematologic toxicity , or if 2 patients experience Grade III nonhematologic and 1 experiences Grade IV hematologic toxicity ( <1 week in duration), no more patients will be treated at that dose level and the maximum tolerated dose will have been exceeded.

C.7.2.2.6 The dose level at which two patients experience Grade III (nonhematologic) toxicity or one patient experiences Grade III (nonhematologic) toxicity and one Grade IV( hematologic and lasting <1 week toxicity), will be classified as the maximum tolerated dose.

C.7.2.2.7 If we reach a situation where the MTD seems to be placed between an extremely safe level and an unacceptably toxic level, we will enroll a cohort of patients at an intermediate dose level according to this schema.

C.7.2.2.8.At the MTD, a total of 30 patients will be treated in order to provide adequate evaluation of radioisotope content of marrow and marrow response at that level and in order to determine tumor response rates. A two stage design is recommended for this part of the study so that the trial can be terminated early if $^{90}Y$ immunoconjugate is not active in this group of patients. Seventeen patients will initially be entered and we assume that at least 15 will be eligible. If one or more responses are observed among the 15 eligible patients, an additional 17 patients will be entered. .

C.7.2.3 In-111/ Y-90 BrE-3 methyl benzyl DTPA administration procedure

For the administration of the initial dose levels of $^{111}In$ and $^{90}Y$ hu BrE-3 methyl benzyl DTPA patients may be outpatients. Patients will be hospitalized in the CRC at Bellevue Hospital if and when the level of administered radioactivity ($^{90}Y$ and $^{111}In$) exceeds permissible limits for outpatients. All administrations will be performed under clinical observation. Hypersensitivity skin testing will not be performed prior to each antibody infusion in each patient. Data from the National Cancer Institute suggests no correlation between local erythema and induration at the site of skin tests and subsequent systemic allergic reactions to mouse antibody infusions (K.Foon, personal communication). Skin testing may also increase the likelihood of sensitization to mouse antigens.

C.7.2.3.1 Infusion

Intravenous tubing will be pretreated with 0.9% NaCI and 1% human serum albumin. After placement of an intravenous line, radiolabeled huBrE-3, at the dose described above,will be infused over the course of one hour depending on rate- dependent side effects in a volume of 250 ml of 1% human serum albumin in normal saline.Vital signs will be taken every 15 minutes during the infusion, and every one hour post infusion until stable. A thorough cardiopulmonary physical examination will be done prior to and at the conclusion of antibody infusion. Medications including

acetaminophen, diphenhydramine, epinephrine, and corticosteroids will be kept at hand for treatment of allergic reactions should they occur. An emergency cart will be at hand.

C.7.2.3.2 Radiation safety precautions will be observed by all personnel :

Gloves will be worn when handling samples of blood, urine, or other body fluids.

Laboratory specimens will be labeled with radioisotope labels.

There are no restrictions to visitors, including hospital personnel related to isotope administration while the total administered dose of radioactivity and/ or external exposure rate remains within radiation safety guidelines.

The radiation safety precautions and health safety precautions established by institutional radiation safety guidelines will be observed.

For outpatient administration, no special precautions are necessary in the patient's home.

Patients will be treated as in-patients when the level of administered radioactivity or the anticipated external exposure rate exceeds the permissible limit for out-patient administration. The patients will be monitored by radiation safety on a daily basis. The patient will be confined to hospital until radioactivity levels as determined by radiation safety permit their discharge.

C.7.3 Duration of treatment

C.7.3.1 Patients without progression and less than dose limiting toxicity after one course of treatment, should be retreated at the same dose of Y-90 BrE-3 methyl benzyl DTPA 4 weeks weeks after the last dose. In-111 labeled BrE-3 methyl benzyl DTPA will be coinfused with the first dose of each repeat cycle of administrations.

C.7.3.2 The following criteria must be met before retreatment:

1. Serum negative for human anti-human BrE-3 antibody
2. Neutrophil count $\geq$ 2500
3. platelets > 100,000
4. pO2 $\geq$ 80 mm Hg on room air

C.7.3.3 If persistent hematologic toxicity at 6 weeks prevents retreatment, patients may be treated at 8 weeks if hematologic recovery has occurred.

C.7.3.4 If Grade III (nonhematologic) or Grade IV (hematologic, <1 wk duration) toxicity occurs in a patient with partial or complete response, the patient may be retreated with half the radioactive dose of Y-90 BrE-3 methyl benzyl DTPA received in the initial treatment dose, at the discretion of the investigator. The mass amount of antibody administered at each dose level will be held constant, however.

C.7.3.5 Patients whose sera test positive for human anti-huBrE-3 antibody may be retreated at the discretion of the investigator.

C.7.3.6 Patients who do not show progression 6 weeks after the second course of therapy and have less than Grade III toxicity may be retreated at the discretion of the investigator. There will be no more than 3 cycles of radiolabeled antibody administered to any one patient.

C.7.4 Off study criteria

C.7.4.1 Progressive disease at 6 weeks on study. ( Patients will be followed for a minimum of 8 weeks for toxicity or until toxicity resolves.) If patients have progressive disease prior to 6 weeks, patients may be removed from the study earlier at the discretion of the investigator.

C.7.4.2 Intercurrent illness which prevents further administration of $^{90}$Y BrE-3 methyl benzyl DTPA.

C.7.4.3 Unacceptable toxicity

C.7.4.4 Decision of patient to withdraw from the study

C.7.4.5 General or specific changes in the patient's condition which render the patient unacceptable for further treatment in the judgement of the investigator.

C.8 Management of toxicity and reporting of Adverse Drug Reactions (ADR's)

C.8.1 Hazards and Protection

C.8.1.1 Radiation

C.8.1.1.1 Hazards

The long term toxicities of intravenous radiolabeled monoclonal antibody therapy are not known. Any of the chronic toxicities associated with external beam whole abdominal irradiation such as bowel or bladder fibrosis, liver dysfunction or peritonitis with adhesions could occur potentially. Similarly, chronic toxicities such as pneumonitis and fibrosis from lung irradiation might occur at these dose rates.

The most likely subacute toxicities which might be expected on the basis of other radiolabeled antibody therapy trials (1,17, 26) are hematologic and include pancytopenia and fever. Other toxicities which might be occur depending on the localization of the antibody include diarrhea and peritonitis. Hematologic toxicities are the major toxicity expected and will most likely determine the maximum tolerated dose for Y-90 huBrE-3 methyl benzyl DTPA. These will be graded according to NCI guidelines. (Please see Appendix 1 )

The amount of radiation exposure with In-111 BrE-3 methyl benzyl DTPA is minimal and is not expected to result in any toxicity. However it is anticipated that the radiation exposure from Y-90 will cause the dose limiting toxicity and most likely will be hematologic.

C.8.1.1.2 Monitoring

CBC's with differential will be monitored weekly for 8 weeks or until counts normalize. Hepatic and renal toxicity will be monitored by blood chemistries. Pulmonary toxicity will be monitored by pulmonary function tests and arterial $pO_2$. Serial physical examinations will be performed to monitor for other organ system toxicity.

C.8.1.2 BrE-3 antibody

C.8.1.2.1 Toxicity associated with the antibody

In the three patients who have received In-111 huBrE-3 to date, no allergic or toxic reactions have been observed by clinical or laboratory criteria. Of the 15 patients who received 5 mCi of In-111 labeled murine BrE-3 (5 each at 10 mg, 50 mg, and 100 mg) at NYU Medical center or U.C. Davis, 2 have shown mild allergic reactions which were treated with oral antihistamines and resolve. An additional patient has shown a slight increase in liver function tests. One showed a slight drop in hemoglobin most likely due to blood drawing.

Of the seven patients who have received $^{90}Y$ BrE-3 at doses of 6.25 mCi/m2 (n=3), 9.25 mCi/m2 (n=3), and 12.25 mCi/m2 (n=1), one episode of flushing during administration, and one patient with a single hive within 24 hours of administration were observed. Hematologic toxicity was observed in one patient at 4 weeks after antibody administration and 3 days after chemotherapy ( Grade 1) and a transient Grade 4 thrombocytopenia was observed in the heavily pre-treated patient at the highest dose level. Pretreatment assays of CFU's in her bone marrow showed very little hematopoietic reserve.

Both Mc5 and KC4G3 are similar murine monoclonal antibodies which bind to an epitope very similar to the one recognized by BrE-3. These have been used more extensively in patients.There has been no unexpected toxicity associated with Mc5 in diagnostic imaging trials. As with other murine monoclonal antibody trials in humans the overall toxicity with Mc5 was mild and explainable in terms of allergic reactions or occasional low grade fever. The allergic reactions included low grade fever, chills, pruritus, occasional rash, and occasional headache. There was no evidence of hepatic or renal toxicity. No change in pancreatic enzymes or thyroid function tests was seen.

No unexpected toxicity has been seen with KC4G3 in either diagnostic imaging or therapeutic trials. In nonsmall cell lung cancer patients, a dose escalation study was performed with unlabeled antibody. 10-500 mg was administered twice weekly for 4 weeks. 3 of 15 patients experienced reactions, but with no long term sequelae. Furthermore, these reactions occurred after repeated administration and total doses of 230, 780 or 2000

mg. This included transient hypotension in a patient who developed high HAMA (IgE and IgG) titers. Acute dyspnea occurred in one patient ( 780 mg). A serum sickness like syndrome occurred in the patient who received 2 g. of antibody. We will be administering significantly lower doses of antibody protein in this protocol.

Repeated administration of murine MoAbs more than 10-12 days apart can result in allergic reactions including serum sickness and anaphylaxis.

HAMA may result from the administration of murine monoclonal antibodies such as BrE-3. We have seen HAMA responses in 4/6 patients evaluated to date. HAHA in response to humanized BrE-3 has been seen in trace amounts at 5 weeks and 3 months after infusion. This will be monitored as detailed under Serial Observations.

C.8.1.2.2 Precautions

The radiolabeled antibody will be subject to quality control procedures including immunoreactivity testing, pyrogen testing (limulus amoebocyte lysate tests), sterility, and ITLC.

The antibody will be infused over a period of 1 hours with trained medical personnel in attendance. Vital signs will be monitored during the infusion and for an hour after. A baseline cardiopulmonary physical examination will be performed prior to and after administration of the radiolabeled antibody. An intravenous line which is placed for administration of the radiolabeled antibody will be kept in place. Antihistamines, epinephrine, and corticosteroids will be kept at hand. There is an emergency cart available in the Division of Nuclear Medicine. Patients with grade 1 or 2 toxicity may continue on study at the discretion of the investigator. In the event of more serious reactions, the antibody infusion will be stopped and treatment with subcutaneous epinephrine, intravenous steroids, respiratory assistance other resuscitative measures will be instituted. No further antibody will be administered.

C.8.2 Reporting of adverse drug reactions

Adverse drug reactions to In-111 or Y-90 BrE-3 methyl benzyl DTPA or the unconjugated, unlabeled BrE-3 antibody will be reported by phone to the Principal Investigator, and IDB (301-496-7957) within 24 hours :

a) All serious toxicity (Grade 3 and 4) which may be due to antibody administration

b) All fatal events

c) First occurrence of any toxicity regardless of grade other than Grade I fever .

d) All grade 4 reactions and patient deaths while on treatment

Written reports to follow within 10 working days to :

Investigational Drug Branch
P.O. Box 30012
Bethesda, Maryland 20824

Previously known Grade 2 and Grade 3 reactions are to be reported to the NCI in writing using the "NCI adverse reactions form for Investigational Agents" within 10 working days.

All adverse reactions will also be reported to the Institutional Review Board.

C.9 Criteria for Response

C.9.1 Methods of malignant disease evaluation

C.9.1.1 Measurable Bidimensional:

Malignant disease measurable in two dimensions by rulers or calipers (metric system) with surface area determined by multiplying the longest diameter by the greatest perpendicular diameter( i.e., metastatic pulmonary nodules, hepatic metastases, lymph nodes, and subcutaneous masses). Malignant disease with sharply defined borders visualized by ultrasonography, computerized axial tomography or magnetic resonance imaging is considered measurable. Repeat studies will be performed at the same pretherapy ( baseline) site(s).

C.9.1.2 Measurable, unidimensional.

Malignant disease measurable in one dimension by rulers or calipers (metric system) (e.g., mediastinal adenopathy, malignant hepatomegaly, or abdominal masses)

Mediastinal and hilar involvement may be measured if a preinvolvement chest radiograph by subtracting the normal mediastinal or hilar width on the preinvolvement radiograph from the onstudy width containing malignant disease ( Alternatively, this may be handled as two-dimensional disease if chest CT is available)

Malignant hepatomegaly may be measured if the liver descends >5 cm below the costal margin by adding the measurements below the costal margins and the tip of the xyphoid.

C.9.1.3 Nonmeasurable, evaluable

Malignant disease evident on clinical (physical or radiographic) examination, but not measurable by ruler or calipers (e.g. lymphangitic or confluent multinodular lung metastases, skin metastases, ascites or pleural effusions known to be caused by peritoneal or pleural metastases and uninfluenced by diuretics, bone scans, gallium scans, deviated or obstructed ureters, or gastrointestinal tract and poorly defined masses by ultrasonography, CT, or MRI).

Photographs should be taken prior to and during therapy to document response of externally visible disease.

Malignant ascites known to be caused by malignant involvement of the peritoneum and uninfluenced by diuretics may be followed by serial abdominal girths measured through a specified fixed point

Serial x-rays of lymphangitic or confluent multinodular lung metastases, pleural effusions or bone metastases should be compared to evaluate response.

Bone and other scintigraphic scans can be used to evaluate response.

Chemical parameters and biologic markers will be measured but will not be used to evaluate response. Normalization of hyperbilirubinemia known to be caused by malignant disease may be used as an evaluable response.

C.9.2 Objective Response Criteria

C.9.2.1 Complete response:

Complete disappearance of all clinically detectable malignant disease for at least four weeks. Bony metastases, radiographically detected prior to therapy, must show normalization or complete sclerosis of lytic metastases with a normal bone scan. If only bone scan is positive, this must show normalization.

C.9.2.2 Partial response:

Greater than or equal to 50% decrease in tumor area for at least 4 weeks without appearance of new areas of malignant disease.

C.9.2.2.1 Measurable, bidimensional

$\geq$ 50% decrease in tumor area or a 50% decrease in the sum of the products of the perpendicular diameters of multiple lesions in the same organ site for at least 4 weeks.

C.9.2.2.2 Measurable, unidimensional

$\geq$ 30% decrease in linear tumor measurement for at least 4 weeks.

A partial response of malignant hepatomegaly has occurred if the sum of the liver measurements below the costal margins, in the midclavicular lines, and the tip of the xyphoid decreases by $\geq$30%.

C.9.2.2.3 Nonmeasurable, evaluable

Definite improvement in evaluable malignant disease estimated to be in excess of 50% and agreed upon by two independent investigators

Serial evaluations of chest x-rays and physical measurements should be documented in the records and by photograph when practical

The response should last for at least 4 weeks.

A partial response of bony metastases occurs if there is a partial decrease in the size of the lesions or decreased density of blastic lesions, lasting for at least 4 weeks.

C.9.2.3 Stable

No significant change in measurable or evaluable disease for a least 4 weeks ( $\geq$ 12 weeks for bony metastases) including:

No significant increase in size of any known malignant disease

No appearance of new areas of malignant disease

This includes decrease in size of bidimensional disease of < 50% or decrease in size of unidimensional disease of < 30% or increase in malignant disease of < 25% in any site.

No deterioration of ECOG performance status $\geq$ 1 level related to the malignancy

C.9.2.4 Progressive disease

Significant increase in size of lesions present at the start of therapy or after a response or appearance of new metastatic lesions known not to be present at the start of therapy or stable objective disease associated with a deterioration in ECOG performance status of $\geq$ 1 level related to malignancy.

C.9.2.4.1 Measurable, bidimensional or unidimensional disease

$\geq$25% increase in the sum of the products of the 2 dimensions of the individual lesions in an organ

$\geq$50% increase in the size of the product of the 2 diameters if only one lesion is available for measurement and was $\leq$ 2 $cm^2$ in size at the start of therapy.

$\geq$25% increase in the sum of the liver measurements below the costal margins and xyphoid.

Appearance of new malignant lesions.

C.9.2.4.2 Nonmeasurable , evaluable

Definite increase in the sum of the areas of malignant lesions estimated to be > 25%

Appearance of new malignant lesions ( e.g. bony metastases)

C.10 Study parameters and serial observations (see Appendix 2):

C.10.1 Initial Evaluation

C.10.1.1 Immunohistochemistry will be performed on biopsy or surgical material from primary tumors or metastases to determine the presence and extent of the expression of BrE-3 antigen. Tumor cells must stain positive for the antigen.

C.10.1.2 Patients will undergo complete medical history and physical examination including height, weight, and performance status.

C.10.1.3 Complete CBC including differential blood count and platelet count, PT, PTT.

C.10.1.4 Electrolytes, total protein, albumin, calcium, phosphorus, glucose, creatinine, uric acid, BUN, alkaline phosphatase, total bilirubin, LDH, SGPT and SGOT.

C.10.1.5 Urinalysis

C.10.1.6 Chest x-ray and other x-rays or scans as clinically indicated to document state of disease. A radionuclide bone scan will be performed to assess extent of bony disease. A chest CT will be performed to assess the extent of lung disease.

C.10.1.7 EKG

C.10.1.8 A serum sample (10-15 cc) will be collected pre-injection, aliquoted, and frozen at -20° C for determination of free antigen . (Other samples will be collected at other times points for storage as shown in the serial observations).

C.10.1.9 Bone marrow aspiration will be performed to provide baseline assessment of bone marrow precursor cells and to assess by PCR for the presence of tumor.

C.10.1.10 If the chest radiograph or chest CT are abnormal, pulmonary function tests including arterial $pO_2$ will be performed to assess baseline pulmonary function.

C.10.1.11 A serum sample will be collected pre-injection, aliquoted, frozen at -20° C and shipped by overnight mail on ice for determination of BrE-3 antigen levels. This will be used for the initial screening.

C.10.2 Serial Observations and Laboratory Monitoring Schedule

At the time of the infusion of the In-111/ Y-90 labeled BrE-3 the following serial samples and measurements will be performed for pharmacokinetics and to make dosimetric estimates:

C.10.2.1 Blood samples will be obtained for analysis of radioactivity and for RIA analysis of BrE-3 clearance 5, 120 minutes, 4, 24, 48,and 72 hours, 4, 5, 6 and 7 days after the first injection. Thereafter, samples

will be obtained 5 minutes, 24 hours, 72 hours and 7 days after the second administration.

C.10.2.2 Urine samples will be collected from O-2, 2-4, 4-24, 24-48, 48-72, 72-96,96-144, 144-192 hours after the first infusion. For subsequent administrations, urine will be collected over the first 24 hours.

C.10.2.3 Complete CBC including differential blood count and platelet count, PT, PTT, electrolytes, total protein, albumin, calcium, phosphorus, glucose, creatinine, uric acid, BUN, alkaline phosphatase, total bilirubin, LDH, SGPT and SGOT, and urinalysis will be performed on Day 6, Day 13, Day 20 or 21, and week 4, 5, 6,7 and 8 weeks after the first administration of the Y-90 labeled antibody or until toxicity resolves.

C.10.2.4 Physical examination will be performed on Day 7, 14 , 21, and 4, 6 and 8 weeks after the first administration of the Y-90 labeled antibody to assess response in evaluable disease.

C.10.2.5 An additional serum sample for HAHA will be obtained at Day 6, Day 13 and 5 weeks after Y-90 BrE-3 administration.

C.10.2.6 The anterior and posterior regional gamma camera images which include the chest, abdomen, and pelvis will be obtained at 2, 24, 48 and/or 72 hours and 8, and, optionally, at 10 days after administration of the Indium-111 radioimmunoconjugate. A large field of view gamma camera fitted with a medium energy collimator will be used. Data for each image will be acquired by gamma camera for 1,000,000 counts or 7.5 minutes, which ever occurs first, and archived for later analysis. Anterior and posterior whole body scans will also be performed. The data will be processed and stored with a dedicated computer which is available in the Nuclear Medicine Department to measure regional uptake of radiolabeled BrE-3 in major organs, tumor, and blood pool at the times designated above using region of interest analysis. In addition, SPECT imaging will be performed at 72 hours and/or 7-10 days post administration.

C.10.2.7 An additional bone marrow aspiration and a core biopsy will be obtained at Day 8 and again, when possible an aspiration at 7 days after the second infusion to assess the possibility of further damage. The % injected dose/gram will be measured using both $^{111}In$ and $^{90}Y$. Progenitor assays(CFU-G, CFU-M, CFU-GM,BFU-E, CFU-GEMM) will be performed and compared to baseline studies. Marrow samples will also be assessed by PCR for tumor cells. The marrow in the biopsy on day 8 will be assessed for $^{90}Y$ and $^{111}In$ content ( %ID/g). The bony portion of the core biopsy will be processed to isolate bone for evaluation of $^{90}Y$ and $^{111}In$ content ( %ID/g). If there is evidence of later hematologic toxicity, an optional fourth bone marrow aspiration will be obtained.

C.10.2.8 When accessible tumor is present, biopsy of tumor will be obtained 6-8 days after administration of the $^{90}Y$ methyl benzyl DTPA BrE-3. This tumor biopsy will be analyzed for % injected dose/g of tumor,

immunohistochemical evidence for antibody and antigen. These biopsies will be performed by Dr. Matthew Harris or Dr. Daniel Roses.

C.10.2.9 Pulmonary function tests including arterial $pO_2$ will be performed prior to any repeat radioimmunotherapy.

## C.11 Methods

### C.11.1 Image Analysis/Pharmacokinetics/Dosimetry Estimates

#### C.11.1.1 Pharmacokinetic Analysis

The pharmacokinetic analysis of labeled and unlabeled monoclonal antibody movement into the tumor and through each patient will provide a manageable and useful summary of the data that are collected. The pharmacokinetic analyses will:

1 ) Provide information concerning the biodistribution of both In-111 labeled methyl benzyl DTPA huBrE-3 and Y-90 labeled methyl benzyl DTPA huBrE-3 for radiation dosimetric estimates based on the MIRD formalism.

2) Provide information concerning the pharmacokinetics of Y-90 methyl benzyl DTPA huBrE-3 and products of its metabolism.

3) Provide a basis for correlating the pharmacokinetics of the In-111 labeled methyl benzyl DTPA BrE-3 and the Y-90 labeled methyl benzyl DTPA BrE-3 to determine the potential for using In-111 labeled chelate conjugated antibody to predict the biodistribution and dosimetry of Y-90 labeled methyl benzyl DTPA BrE-3.

In general, the efficiency of elimination of the monoclonal antibody via the urine, and in toto from the blood, will be described using renal antibody clearance and total antibody clearance from the blood, respectively. Uptake of the radiolabeled antibody by the organ(s) of interest will be described over time. The clearances and uptake will provide information which allows an approximation of the biodistribution of the antibody and the radioisotopes. This will most likely relate to the formation of antigen-antibody complexes in the blood as well as the stability of the two different labels on the antibody . Specifically, the estimates based on this information will be correlated with direct measurements of uptake in marrow and, when possible, tumor. These estimates will also be related to the effect on marrow function.

4) Provide a basis for comparing the blood pharmacokinetics of Y-90 MX-DTPA huBrE-3 over repeat administrations.

##### C.11.1.1.1 Biodistribution

In order to study the distribution of conjugated monoclonal antibody labelled with Indium-111, multiple regional scintiphotos will be obtained at 2, 24, 48, and/or 72 hours and one week after injection in the Nuclear

Medicine Department. Regions of interest will be established and counts per pixel calculated for those organs which have significant uptake and for a background region in order to calculate relative uptake ratios. Previous studies have demonstrated significant accumulations of radioactivity in liver, spleen, kidneys, and lungs. In addition, serial blood sampling will provide an estimate of whole body distribution of radiolabel.

Attenuation of activity will be corrected for by transmission images obtained just prior to antibody administration for each patient using a flat field source filled with a known concentration of $^{111}In$. Alignment of digital transmission and emission images will be performed using fiduciary markers and a two-dimensional image registration algorithm within *qsh*, an image handling toolkit which runs under a UNIX operating system (53). Whole body counts will be obtained from anterior and posterior whole body scans. Activity within an organ will be determined using the attenuation corrected geometric mean of conjugate views (60). The consistency of detection sensitivity of the camera will be checked on a daily basis at the time of the patient imaging by imaging a standard at a fixed distance to yield a system calibration factor (14). This standard will be used to convert region of interest data into absolute amounts of radioactivity and also to express activity within regions of interest as percent injected dose. From this data, the fraction of the radiolabel resident in each organ can be closely estimated.

For estimation of tumor radiation absorbed dose, the uptake will be calculated from the attenuation-corrected geometric mean if the localization is observed on both anterior and posterior views . If the tumor localization is observed on only one view, the activity will be calculated based on the effective point source assumption(66).

The standard MIRD formulation will be used to calculate the radiation absorbed dose based on the calculated activity localized either in normal organs or tumor.

C.11.1.1.2 Evaluation of Urine/Plasma

In general, the goal of these studies is to provide better understanding of the biodistribution of Y-90 labeled methyl benzyl DTPA BrE-3 and to better understand the similarities and differences between the In-111 and Y-90 when infused labeled to this chelate conjugated antibody so that eventually the In-111 labeled compound may be used to predict the behavior of the Y-90 labeled compound. For each first infusion of radiolabeled monoclonal antibody, blood samples will be drawn at 5 minutes ,2, 4, 24, 48, and 72 hours, 4, 6, and 8 days post infusion. Urine samples will be collected from O-2, 2-4, 4-24, 24-48, 48-72, 72-96,96-144, 144-192 hours postinfusion. For subsequent infusions, urine will be collected over the first 24 hours and blood samples will be obtained at 5 min. after the end of infusion, 24 hours, 72 hours and 6 days.

Y-90 and In-111 labeled antibody will be measured in serial serum samples and in urine to provide estimates of whole body distribution of the radiolabel. Time activity curves will be generated and half-times calculated.

Total radioactivity (dpm/ml) will be measured in each serum sample and urine sample. We will count aliquots of blood and urine obtained at multiple time points in comparison to a standard made from the administered dose. This is then expressed as % injected radioactivity cleared or excreted over time, allowing for normal radioactive decay by standard decay formulas. In addition, HPLC will be performed on plasma samples to determine the form in which each radioisotope exists, specifically free isotope, chelated, antibody bound, or transchelated ( especially In-111). This data will be used in the pharmacokinetic analysis.

C.11.1.1.3 Blood pharmacokinetics

The distribution of monoclonal antibody will be followed by radioactivity determinations from the timed blood samplings. These will be converted into monoclonal antibody concentration based on the specific activity determination of the labeled BrE-3 antibody . Nonlinear pharmacokinetic models which run on a PC , PCNONLIN (Statistical Consultants, Lexington Ky) will be used for the analysis of the data. Previous studies with "first generation" monoclonal antibody imaging reagents have shown that postinfusion blood concentrations can follow either patterns that resemble one or two compartment models that reflect both the dose and the physiologic characteristic of the patient. Indeed, analysis of In-111 BrE-3 has shown a monoexponential clearance from serum. Initial parameter estimates will be obtained using the JANA curve stripping programs (Statistical Consultants, Lexington Ky). These estimates will be applied to the appropriate model to best fit the time/concentration MAB data with respect to the duration of the infusion as well as the IV route of administration. The nonlinear models in PCNONLIN use LaGrangian techniques to reduce the total variance of appropriate parameters and yield the key pharmacokinetic parameters. These include half-lives for each compartment (e.g. alpha, and beta for 2 compartment models), the area under the plasma concentration versus time curve (AUC), the apparent volume of distribution, intercepts ( e.g. A & B in a 2 compartment model). The time of distribution , total body clearance, and renal clearance are derived using urine excretion data. The clearance values will reflect the volume of blood and or urine from which the monoclonal antibody is removed per unit time (ml/min).

C.11.1.1.4 Dosimetry calculations

Organ data for the In-111 labeled methyl benzyl DTPA huBrE-3 will be obtained from the scans using region of interest analysis, attenuation correction of data, and the geometric mean of the anterior and posterior views of each organ.

The blood and organ radioactivity data will be examined using a computer program "S" designed to fit the data to a curve or curves. This program provides estimates of slopes and intercepts for each exponential component. The slopes and intercepts for the exponentials will be used with the standard

MIRD dosimetry formulations (as implemented by MIRDOSE2, Oak Ridge) to make dosimetric estimates.

Excretion rate plots (urinary excretion rate of radioactivity vs. time) will be examined in a manner similar to the analysis of the blood radioactivity data.

Finally, when possible, we will use the data obtained from imaging studies from the tumor site to estimate tumor dosimetry. Count density from the scans will be used to estimate change in tumor uptake over time. More helpful will be data obtained if and when accessible tumor is biopsied. This can be compared with scan data to develop dosimetric estimates.

C.11.1.2 Analysis of Marrow

Bone marrow aspiration will be performed prior to infusion and at Day 8. Marrows will be counted for both Y-90 and In-111. Aspirates will be evaluated with marrow progenitor assays (BFU-E, CFU-G, CFU-M, CFU-GM, CFU-GEMM). Aspirates will also be submitted for PCR to evaluate for tumor cells.Bone marrow biopsies will be evaluated for marrow content of radioactivity. A portion of the bone marrow biopsy will be purged of bone marrow and then counted for radioactivity.

Radioactivity concentrations in marrow will be examined in relation to activity measured from scans over vertebral regions of interest and to measured blood activity.

C.11.1.3 Analysis of Biopsies

In patients with accessible tumors, when feasible, we will biopsy tumor 6-8 days after infusion of radiolabeled antibody to evaluate DPM.'s/gram tissue weight and immunohistochemical analysis of tumor antigen expression and uptake by antibody.

C.11.1.4 Image evaluation

The categorical evaluation of the radionuclide scans will be scaled based on the ratings of two observers. This will be performed for each patient and for each known lesion.

C.11.2 Determination of circulating BrE3 antigen

Circulating BrE-3 epitope in the serum of treated patients will be determined by a competitive serum assay with the BrE-3 epitope on the solid phase. Microtiter plates precoated with a source of BrE-3 epitope will be presented with stoichiometric quantities of BrE-3 and the serum added in adequate dilution. After an overnight incubation BrE-3 bound to the solid phase will be detected by a radioiodinated anti-mouse Ig antibody. Results obtained will be compared to a standard curve originated against increasing concentrations of the epitope and expressed as µg/ml of protein equivalent breast epithelial mucin.

BrE-3 antigen levels will be performed at the John Muir Cancer Center in Dr. Ceriani's laboratory. Samples will be sent by overnight mail and processed immediately.

C.11.3 HPLC Analysis of Urine and Plasma

The analysis by gel permeation chromatography of plasma and urine samples will allow the assessment of the degree of labeled antibody and free label as well as possible immune complexes at the various time points followed during the pharmacokinetic sampling. Samples will be applied to a 7.8 mm by 600 mm BioSil SEC-250 column equilibrated with 50 mM phosphate buffer and 0.1M $Na_2SO_4$ pH 6.8 with a flow rate of 0.6 ml/min. Detection will be at 280 nm and 0.6 ml fractions will be collected for determination of radioactivity. BioRad molecular weight callibration standards (ribonuclease, ovalbumin, myoglobin, gamma globulin, and thyroglobulin) will be used.

Analysis by HPLC will determine levels (specific activity) of serum and/or urine $^{111}In$ labeled BrE-3 and $^{90}Y$ labeled BrE-3.

C.11.4 Immunohistochemical analysis of biopsied tumor samples and previously obtained tissue for screening.

Immunohistochemical staining is performed under Dr. Howard Mizrachi's supervision with a full time registered histology technician performing all antibody staining. Tumor tissue will also be subject to routine histopathologic staining. Histopathology of previously obtained tumor specimens for eligibility screening and examination of any tumor biopsies will include:

Gross examination of freshly obtained tissue:

1. Site.
2. Size

Microscopic evaluation will include:

1. Histologic subtype.
2. Histologic grade.
3. Nuclear grade.

Immunohistochemical evaluation will include:

1. % of cell positive ( 0 to 100%)
2. Intensity (0 to 3+)

Control will be evaluated for:

1. % of cell positive (0 to 100%)
2. Intensity (0 to 3+)

C.11.5 Histopathological examination of bone marrow

Bone marrow aspirates will be assessed histologically for cellularity and for the presence of metastatic carcinoma. Cellularity will be expressed as the percentage of the cellular marrow elements in relation to the total of the cellular elements and the bone marrow fat. Cellularity normally decreases with age. The results will also be reported semiquantitatively as normocellular, slightly hypercellular, markedly hypercellular, slightly hypocellular, markedly hypocellular, or acellular.

C.11.6 Immunoreactivity

The degree of immunoreactivity will be assessed after each radiolabeling procedure. The assay employs a single radiolabeled antibody concentration that is coupled with the use of antigen-coated beads to produce a determination the amount of non-specific binding along with the percent of total binding. The assay utilizes negative controls which consist of bovine serum albumin coated beads . A brief description of the protocol for immunoreactivity testing follows below:

Reagents: 1% BSA RIA grade (Sigma) in 10 mM potassium phosphate buffered 0.9% sodium chloride containing 0.1% sodium azide, pH 7.2 (1% BSA-PBS), wash buffer consisting of 0.15M Sodium chloride, 10 mM phosphate, 0.,1% azide, 0.05% Tween 20. Beads (6.4 mm polystyrene, Precision Ball Co) coated respectively with albumin for non-specific binding (NSB) and antigen for specific binding (Bmax).

Protocol: Radiolabeled antibody is diluted to yield the equivalent of 25,000 cpm per ml in assay buffer. Tubes are prepared to contain in triplicate the NSB, the Bmax and the total counts (TC). To each tube with the exception of the total counts, 0.2 ml of the diluted radiolabeled antibody is added. After incubation overnight at room temperature, the solution is aspirated from each of the NSB and Bmax tubes followed by the addition of 3 ml of the wash buffer. The wash buffer is aspirated off and the tubes including the TC are counted in a gamma counter. The labeling is considered acceptable at values of 50% binding or better with less than 3% non-specific binding and a CV for triplicate analyses of each sample.

C.11.7 HAHA

C.11.7.1 IgG

Anti-human IgG and antiidiotype are determined by mixing radiolabeled huBrE-3, or matched isotype human IgG with the patients serum . After incubation, samples are processed on an HPLC column which gives sufficient resolution to identify Ab-Ab complexes.. The bacteria are then washed and counted for determination of anti-mouse IgG.

C.11.8 Hematopoietic progenitor assays

These assays will be performed on all marrow aspirations obtained.

Samples will be collected into preservative free heparin (100 u/ml), mixed well, and gently expelled into disposable, sterile, screw-topped vials. Samples are held on ice until delivery to the core laboratory.

Marrow samples are diluted 4 to 5-fold with RPMI 1640 + 10% heat-inactivated calf serum. 8 ml of the diluted sample are then layered over 4 ml of Ficoll-Hypaque for density centrifugation. Cells at the interface are removed, diluted gently with medium, centrifuged and washed. Cells are suspended in IMDM + 10% heat-inactivated fetal calf serum and counted. Cytospin preparations are made of the cell inocula for subsequent examination if warranted. Cells are suspended to a

concentration of 1 X $10^5$ cells/ml for marrow. Aliquots of 0.4 ml of cell are then added to 4 ml of freshly thawed plating medium. These mixtures are vortexed and plated into bacteriological dishes (1ml/ dish; 3 dishes per sample). The cultures are incubated in a well-humidified chamber at 37°C for 12-14 days and read for colony formation. Colonies are distinguished on the basis of established criteria. Cytospin preparations are made of representative colonies from each sample series to verify identifications.

C.12 Statistical Considerations

D.12.1 Duration of Study : We expect to enroll a total of 46 patients in this Phase I/II study; however, the maximum sample size is 52 patients. It is expected that 3 patients will be accrued every 7 weeks during the dose escalation phase of the study. We may be able to repeat therapy in a few patients. Although the duration of the study will depend on the number of doses investigated and whether additional patients are required at one or more levels, the Phase I dose excalation study should be terminated within 12-13 months.

D.12.2 Data analysis : As demonstrated in Appendix 3 several outcomes will be measured in this study. For the dose escalation portion of the study, the results of this study are not meant to be definitive but, rather to gain information for future plans. For this reason, formal statistical inference testing will not be performed on these data; however, a number of descriptive analyses will be presented.

The major clinical endpoints of this study will be toxicity initially. The MTD is that dose at which one third of patients experience dose limiting toxicity (DLT). Escalations are planned in groups of three patients, with an additional three patients to be added at the first indication of the MTD.Three patients will be studied at the first dose level. All three patients must be studied for at least 6 weeks. If none of these three patients experience DLT, the treatment dose will be escalated to the next higher level in the three subsequent patients. If one of three patients experience DLT at a given dose, three more patients will be added at the same dose. If none of the three additional patients experience DLT, the dose will be escalated for the next patient treated. If two patients of the total six patients at a given dose level experience DLT, then this dose will be classified as the MTD. Once three patients experience Grade III (nonhematologic) or two patients experience Grade III ( nonhematologic) and one has Grade IV(hematologic, <1 week's duration) at a given dose level, then the MTD will have been exceeded. Similarly, if one instance of Grade IV (hematologic > 1 week's duration or nonhematologic) occurs, then the MTD will have been exceeded. A total of 6 patients will be studied at the preceding dose level.

At the lower doses, this study may generate preliminary information about therapeutic efficacy. Levels and duration response will be tabulated for each patient treated below MTD.

At MTD, a two stage design will be used for the Phase II efficacy study so that the trial can be terminated early if $^{90}Y$ immunoconjugate is not active in this group of patients. Seventeen patients will initially be entered and we assume that at least 15 will be eligible. If one or more responses are observed among the 15 eligible patients, an additional 17 patients will be entered. The chance that the trial will be stopped early, i.e., no responses in the first 15 eligible patients, is less than 4% if the true response rate is at least 20%.

The table below provides 90% confidence intervals for the true but unknown response rate of $^{90}Y$ immunoconjugates given possible observed response rates and assuming that the trial dose continue to a total of 30 eligible patients. For example, if 9 responses are

observed ( observed response rate = 30%), the 90% confidence interval for the true response rae is 17% - 47%.

| Observed Interval Number of Responses rate | Observed Response Rate | 90% Confidence for the True but unknown Response |
|---|---|---|
| 6 | 20% | (9%,36%) |
| 9 | 30% | (17%,47%) |
| 12 | 40% | (25%,57%) |
| 15 | 50% | (34%,66%) |

The table below provides 90% confidence intervals for the true but unknown rate of occurrence of Grade 3 or worse complication, assuming that the trial does ncontinue to a total of 30 eligible patients. For example, if 3 patients experience a particular severe or worse complication ( observed rate = 10%), the 90% confidence interval for the true rate of that particular complication at a grad of 3 or worse is 3-24%.

| Observed Number of Complications | Observed Complication Rate | 90% Confidence Interval for the True but unknown Complication rate |
|---|---|---|
| 0 | 0% | (0%,9%) |
| 1 | 3% | (0%,15%) |
| 2 | 7% | (1%,20%) |
| 3 | 10% | (3%,24%) |
| 4 | 13% | (5%,28%) |
| 5 | 17% | (7%,32%) |
| 6 | 20% | (9%,36%) |

The serial serum and urine measurements are taken at 5 minutes post infusion, 2 hours, 4 hours, 24 hours, 48 hours, 72 hours , at days, 5, 6, and 8. For each patient the measurements from each source will be plotted over time in order to assess 1) how the relation between $^{111}In$ and $^{90}Y$ varies over time for each source of both measurements; and 2) whether the functions of time are consistent over the patients. Graphical displays will also be used to determine whether the half-time clearance of radioactivity has a

consistent relation between $^{90}Y$ and $^{111}In$ over patients for serum and urine measurements. For subsequent infusions only $^{90}Y$ will be measured at the end of infusion, 24 hours, 72 hrs and Day 6. These measurements will be plotted over time in order to assess 1) how the $^{90}Y$ varies from the first infusion to the second or third and 2) whether functions of time are consistent over the patients.

Pharmacokinetic parameters for $^{111}In$- labeled antibody and for $^{90}Y$-labeled antibody will be calculated and will include: peak plasma level (C max), time to peak plasma level (t max), plasma elimination half-life (t 1/2), area under the plasma concentration-time curve (AUC). These parameters for $^{90}Y$- and $^{111}In$- labeled antibody will be tabulated for each patient.

The gamma camera imaging is performed for $^{111}In$ at 2, 24, 48, and/or 72 hours, at day 8, and optionally, at day 10. The images will be analyzed using region-of-interest analysis for organ uptake of $^{111}In$ radioactivity. For each patient these measurements from each organ will be plotted over time in order to assess whether the functions of time are consistent over the patients.for organ distribution.

Radioactivity levels for both $^{111}In$ and $^{90}Y$ will be derived at day 8 from serum, urine, marrow aspirate, marrow biopsy and for $^{111}In$ by gamma camera imaging. These measurements will be graphically presented in order to 1) determine whether an obvious relation exists over all patients between the $^{111}In$ value and the corresponding $^{90}Y$ value from the same source; 2) to explore possible relations among the five $^{111}In$ measurements over all patients; 3) to ascertain whether the same relations exist among the subset of four $^{90}Y$ measurements. These graphical displays, along with the relevant set of Kendall's correlation coefficients, will give an indication of whether the $^{111}In$ labeled compound can be used to predict the activity of the $^{90}Y$ labeled compound, specifically at day 8 and whether the blood (serum) levels can be used to predict bone marrow levels of radioactivity specifically at day 8. Correlation coefficients will be calculated using data from all patients, regardless of the dose level.

Baseline values of and percent changes in CFU's will also be tabulated for all five precursors for each patient. The Wilcoxon signed-rank test will be performed for each CFU assay in order to assess whether significant changes have occurred indicating toxicity. However depending on sample sizes, meaningful changes may not be supported by statistical significance. Changes in CFU's will be correlated with levels of accumulated $^{90}Y$ levels in the marrow and bone and with radiation dose estimates by means of graphical displays and Kendall's correlation coefficients.

Radionuclide scan findings will be tabulated for each patient based on the ratings of two observers. This will be done on a per patient basis and for known lesions, on a per lesion basis.

The analysis for safety will include examination of all changes in physical examination and laboratory evaluation. All clinical and laboratory parameters will be monitored for safety according to NCI toxicity guidelines ( Appendix 1). All adverse experiences reported during study therapy will be tabulated.

C.13 Data Management

Weekly meetings of the co-principal investigators will be held to assess data, logistics, and coordinate the group's effort. Monthly meetings of all investigators and support staff will be held to review progress and potential problems. Dr. Ceriani will be consulted on a monthly basis to report on progress of the NYU group and coordinate with the direction and progress of the overall program. Data will be transferred to Dr. Ceriani on a biweekly basis via modem.

## D. Potential risks

D.1 Monoclonal antibody

Human administration of BrE-3 has been very limited to date. We have administered between 10 and 100 mg of BrE-3 ( 2mg of the methyl benzyl DTPA chelate conjugate form with 8 mg non-conjugated) labeled with 5 mCi of $^{111}In$ to a total of 15 patients to date. Two patients experienced mild, transient allergic reactions. One patient with a large amount of metastatic liver disease experienced a transient Grade 1 elevation of liver function tests. In one patient a 1 gm drop in hemoglobin was observed, most likely due to blood drawing.

Both Mc5 and KC4G3 are similar murine monoclonal antibodies which bind to an epitope very similar to the one recognized by BrE-3.

There has been no unexpected toxicity associated with Mc5 in diagnostic imaging trials. As with other murine monoclonal antibody trials in humans the overall toxicity with Mc5 was mild and explainable in terms of allergic reactions or occasional low grade fever. The allergic reactions included low grade fever, chills, pruritus, occasional rash, and occasional headache. There was no evidence of hepatic or renal toxicity. No change in pancreatic enzymes or thyroid function tests was seen.

No unexpected toxicity has been seen with KC4G3 in either diagnostic imaging or therapeutic trials. In nonsmall cell lung cancer patients, a dose escalation study was performed with unlabeled antibody. 10-500 mg was administered twice weekly for 4 weeks. 3 of 15 patients experienced reactions, but with no long term sequelae. Furthermore, these reactions occurred after repeated administration and total doses of 230, 780 or 2000 mg. This included transient hypotension in a patient who developed high HAMA (IgE and IgG) titers. Acute dyspnea occurred in one patient ( 780 mg). A serum sickness like syndrome occurred in the patient who received 2 g. of antibody.

We will be administering significantly lower doses of antibody protein in this protocol.

The radiolabeled antibody will be subject to quality control procedures including immunoreactivity testing, pyrogen testing (limulus amoebocyte lysate tests), and ITLC.

The antibody will be infused over a period of 2 hours with trained medical personnel in attendance. Vital signs will be monitored during the infusion and for an hour after. A baseline cardiopulmonary physical examination will be performed prior to and after administration of the radiolabeled antibody. An intravenous line which is placed for administration of the radiolabeled antibody will be kept in place. Antihistamines, epinephrine, and corticosteroids will be kept at hand. There is an emergency cart available in the Division of Nuclear Medicine. Patients with grade 1 or 2 acute toxicity may continue on study at the discretion of the investigator. In the event of more serious reactions, the antibody infusion will be stopped and treatment with subcutaneous epinephrine,

intravenous steroids, respiratory assistance other resuscitative measures will be instituted. No further antibody will be administered.

D.2 Radiation

Biodistribution of $^{111}$In labeled methyl-benzyl DTPA-BrE-3 monoclonal antibody has been studied in 15 patients with metastatic or recurrent breast carcinoma. Biodistribution an organ pharmacokinetics have been used to develop radiation dosimetry estimates for $^{111}$In labeled methyl-benzyl DTPA-BrE-3. These have also been extrapolated to 90Y-MX-DTPA BrE-3. . The average whole body dose from 111 In MX DTPA is estimated to be 0.45 rads/mCi administered and from $^{90}$Y-MXDTPA BrE-3 the estimated whole body dose is 2.14 rads/mCi administered. The marrow doses for $^{90}$Y-MXDTPA BrE-3 ranged from 0.44 rads/mCi to 14 rads/mCi administered.

D.2.1 Radiation Safety/Precautions

Gloves will be worn when handling samples of blood, urine, or other body fluids.

Laboratory specimens will be labeled with radioisotope labels.

There are no restrictions to visitors, including hospital personnel related to isotope administration while the total administered dose of radioactivity and/ or external exposure rate remains within radiation safety guidelines.

The radiation safety precautions and health safety precautions established by institutional radiation safety guidelines will be observed.

For outpatient administration, no special precautions are necessary in the patient's home.

Patients will be treated as in-patients when the level of administered radioactivity or the anticipated external exposure rate exceeds the permissible limit for out-patient administration. The patients will be monitored by radiation safety on a daily basis. The patient will be confined to hospital until radioactivity levels as determined by radiation safety permit their discharge.

A history of contraceptive use or a negative pregnancy test will be required of all women of child-bearing potential entered in this protocol.

D.3 Venopuncture:

A total of 100 ml of whole blood will be drawn over a period of a week for pharmacokinetics. The early time point will be drawn through a small peripheral intravenous line to minimize the number of needle sticks. In addition, approximately 25 ml will be required for routine CBC and chemistry as part of the initial evaluation and again for each weekly follow-up for 6- 8 weeks.

Venopuncture and blood drawing will be performed by experienced medical personnel. All laboratory specimens will be labeled with radioisotope labels. Efforts will be made to minimize the number of punctures necessary to obtain the requisite blood samples.

D.4 Confidentiality:

The records of the subjects entered into this study will be kept in a locked file. Beyond the study personnel, only representatives of the FDA or Coulter Immunology will have access to these files.

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